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Non-metric Multidimensional Scaling of Barberry (*Berberis* spp.) Genotypes: Insights from Morphological, Nutritional, and Biochemical Data

Mahsa Khodabandeh[1] · Majid Azizi[1] · Ahmad Balandari[2] · Seyedeh Faezeh Taghizadeh[1,3]

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Abstract

Barberry (*Berberis* spp.) is considered beneficial in the treatment of metabolic abnormalities. Tiny barberries with unique taste and flavor make them ideal for the food industry. This study investigated the morphological, nutritional, and biochemical traits of 12 *Berberis* genotypes. Non-metric multidimensional scaling (NMDS) and principal component analysis (PCA) were used to classify the genotypes. Based on the results, genotypes #8-3 and #2-2 showed the highest length and width of berries, respectively. Pulp percentages ranged from 51.93 to 98.5%. The lowest fresh and dried weights of 100 berries were found in #11-1 and seedless, respectively. The highest levels of ascorbic acid and protein contents were obtained in #14-2. The potential genotype for total soluble sugar (TSS) was seedless (59.61%). Genotype #10-1 showed the fiber content and average nutritive value (ANV) with the highest levels of Fe and Ca. Genotype #5-1 showed the maximum amounts of total phenolic compound (TPC), while the highest levels of total flavonoid compound (TFC) and total monomeric anthocyanin content (TMAC) were related to #14-2. Consequently, the IC_{50} value of #14-2 was significantly lower than the other genotypes. Genotype #14-2 with a unique black color and a bluish wax showed 29.44, 2.31, and –3.23 lightness (L*), greenness [–] to redness [+] (a*), and blueness [–] to yellowness [+] (b*), respectively. In terms of TPC, the results of the NMDS ordination showed the separation of #12-1, while the TFC and TMAC results showed the separation of #14-2. The stress values were low, which indicated the high goodness of fit of the ordination distances to the observed distances.

Keywords Barberry · Multivariate ordination · Natural products · Phytoconstituents

Introduction

Barberry (*Berberis* L.) belongs to the Berberidaceae family (Pinar et al. 2021). This family contains 14 genera and approximately 700 species that are found in Asia, Europe, and America (Christenhusz and Byng 2016). The co-existence of different evergreen, semi-evergreen, and deciduous barberries leads to an exceptionally complex vegetation collection in Iran. Commonly, barberry shrubs are 1–3 m in length, with yellow wood and diverse fruit colors (Sarraf et al. 2019).

The well-known seedless cultivar is mostly red and wild seeded could be found in crimson, purple, wine red, orange, and black. Some of the species are covered with a thin waxy layer, which gives them an opaque appearance or a bluish hue in black types. The seeded and seedless barberries also have different flavors. The seedless barberry has a mildly sour taste, while the wild types have different tastes like the astringent or citrus-like tastes (Alemar-

The authors Mahsa Khodabandeh and Ahmad Balandari contributed equally to the manuscript.

Data Availability Statement The data that support the findings of this study are available from the corresponding author upon reasonable request.

✉ Majid Azizi
azizi@um.ac.ir

✉ Seyedeh Faezeh Taghizadeh
taghizadeh-f@ferdowsi.um.ac.ir

[1] Department of Horticultural Sciences, Faculty of Agriculture, Ferdowsi University of Mashhad, P. O. Box 9177948978, Mashhad, Iran

[2] Department of Food Quality Control and Safety, Research Institute of Food Science and Technology, Mashhad, Razavi-Khorasan Province, Iran

[3] Pharmaceutical Research Center, Pharmaceutical Technology Institute, Mashhad University of Medical Sciences, Mashhad, Iran

dan et al. 2013). In traditional medicine, *Berberis vulgaris* has been widely used for treating diarrhea, gastroenteritis, and kidney stones. Several pharmacological studies showed that the different parts of this plant have numerous therapeutic effects in treatment of various diseases, including metabolic syndromes (e.g. type II diabetes mellitus, obesity, hypertension, and dyslipidemia), and polycystic ovarian syndrome. The antioxidant, antimicrobial, mutagenic, anti-leishmaniasis, anti-inflammatory, anti-cancer, and anticholinergic properties of barberry are those the most studied in various clinical trials (Imenshahidi and Hosseinzadeh 2019; Nakamura et al. 2003). The fruits have many other folk applications including protection against colitis, treatment of seizures, epilepsy, and high blood pressure. Indians used barberry fruit as an appetite tonic and gargle (Fatehi et al. 2005; Kulkarni and Dhir 2008).

Since Iran is one of the greatest plant diversity centers of barberry, the organized collection of the Middle East has been established in Mashhad, Razavi-Khorasan province, Iran. Variation among species, genotypes, and environmental/geographical factors result in the content of the secondary metabolites in different barberries, which should be taken into consideration when applying these plants as therapeutic and food products (Farhadi et al. 2020). Many scientists in Iran are currently exploring the phytochemical contents of various parts of barberry (Mokhber-Dezfuli et al. 2014). However, there is no report in the literature to visualize differences in secondary metabolite profiles among the genotypes. For this purpose, we used the mathematical model for analyzing phytochemical turnover among different genotypes. Non-metric multidimensional scaling (NMDS) is a robust multivariate ordination method that computes the two-dimensional locus for each individual variable (Wang et al. 2013). This could also be detected by principal component analysis (PCA) (with normalization), but NMDS is crisper than PCA. The NMDS algorithm works on the binary relations among the objects. This certain linear method could provide sensible results with less computational effort (Mohammadi et al. 2021; Taguchi and Oono 2005).

The current study presents: (i) morphological traits; (ii) nutritional traits including ascorbic acid content, total protein content, dietary fiber content, total soluble sugar (TSS), element content, and average nutritive value (ANV); (iii) biochemical traits including total phenolic compound (TPC), total flavonoid compound (TFC), total monomeric anthocyanin content (TMAC), antioxidant assay (2, 2-diphenyl-1-picrylhydrazyl [DPPH] and ferric reducing antioxidant power [FRAP]), and color determination of 12 barberry genotypes from Iran; (iv) classification of the selected genotypes by multivariate analysis.

Materials and Methods

Plant Materials

The fruit clusters and shrubs of the 12 barberry genotypes were collected from South-Khorasan province, Iran (Fig. 1). Selection criteria for barberry genotypes were usability based on shape, size, and color. The ripe fruits of 17-year-old shrubs were hand harvested randomly from four directions of each shrub. In order to determine the variations, the fruits were categorized as fresh and air dried, then they stored at 4 °C until further analyses. The soil features and climate conditions of the collection zones are presented in Tables 1 and 2, respectively.

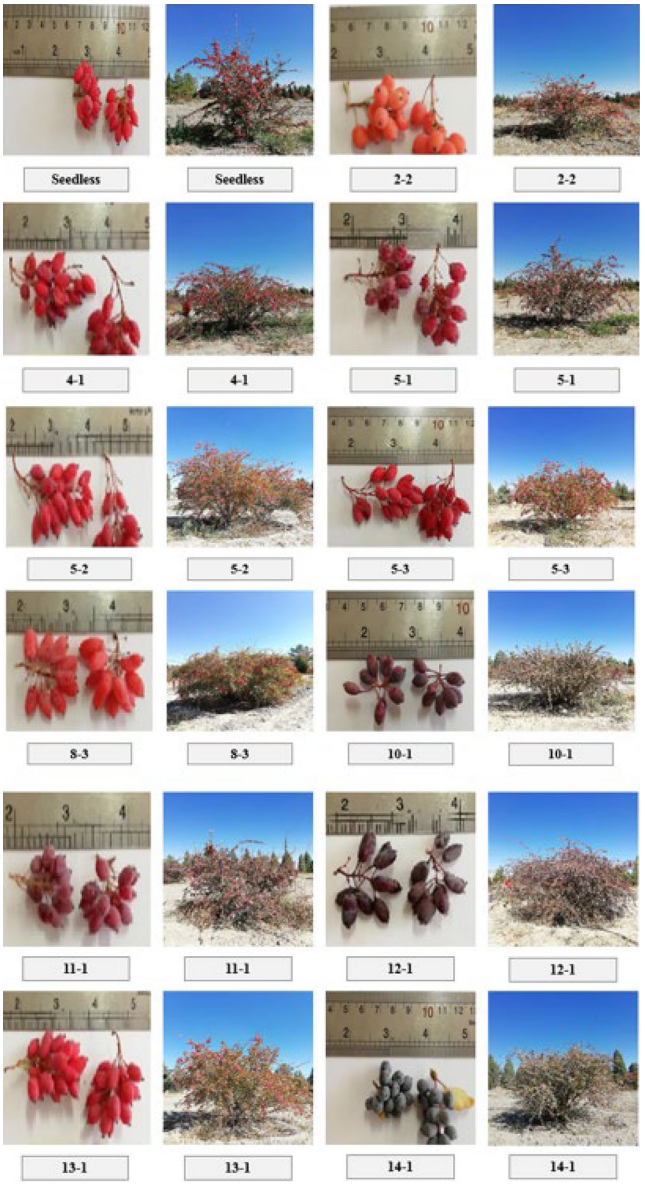

Fig. 1 Fruit clusters and shrubs of the evaluated barberry genotypes

Table 1 Soil parameters of the collection zones

Parameter	Sample depth (cm)	
	0–30	30–60
Sand (%)	52.00	64.00
Silt (%)	34.00	24.00
Clay (%)	14.00	12.00
pH	8.02	7.87
EC (ds/m)	1.95	1.30
SP (%)	31.70	29.71
TNV (%)	12.32	11.20
N (%)	0.051	0.02
P (mg/kg)	14.60	11.20
K (mg/kg)	270.00	135.00
OC (%)	0.56	0.28

EC electrical conductivity, *SP* saturation percent, *TNV* total neutralizing value, *OC* organic carbon

Table 2 Climate parameters of the collection zones

Parameter	Value
Climate	Semi-arid
International Civil Aviation Organization code	40745
Longitude (E)	59°22′
Latitude (N)	32°87′
Elevation (meters above mean sea level) (MAMSL)	999.20
Average annual minimum temperature (°C)	7.10
Average annual maximum temperature (°C)	21.10
Average annual temperature (°C)	14.00
Average annual vapor pressure	8.27
Average annual relative humidity (%)	55
Average annual precipitation (mm)	257.43
Dominant wind direction	West to East
Total annual sunshine duration (h)	2500

Morphological Analysis

To determine the cluster morphological traits, 10 clusters were randomly selected from each replication. The length and width of the berry, percent of pulp, numbers of non-aborted and aborted seeds, as well as the fresh and dried weights of 100-berrry samples were determined. In order to determine the percentage of fruit moisture, the samples were incubated at 75 °C for 48 h. They were then weighed and compared with their pre-incubation weights in the case of weight loss; heating was repeated until the weight was fixed. Moisture (%) was determined according to Eq. 1 (Rahmati et al. 2018):

$$Moisture\ (\%) = \frac{W1 - W2}{W1} \times 100 \quad (1)$$

- W1: Initial weight
- W2: Final weight

Nutritional Analysis

Ascorbic Acid Content

In order to measure the ascorbic acid, dried fruits were incubated in an oven at 35 °C for 24 h. Then, 100 mg of each sample was weighed in a plastic tube, and then 5 mL of 5% meta-phosphoric acid was added. The samples were shaken and extracted in an Elma® (Germany) ultrasonic bath (30 kHz, 25 min, and 30 °C) (Aminifard et al. 2012b). After this time, the samples were centrifuged at 6000 rpm for 10 min. The supernatant was transferred to a clear high performance liquid chromatography (HPLC) vial and 20 µL was used for injection. Chromatograms were obtained via a Knauer® HPLC equipped with a smart line® pump (model 1000) and a smart line® UV detector (model 2500) at 235 nm. The column was a VDS optilab® C18 (250 × 4.6 mm). The mobile phase (in isocratic elution) was composed of 525 mL of solution A and 375 mL of solution B. (solution A: 20 mL glacial acetic acid was added to 0.96 g sodium 1-pentanesulphonate; solution B: 20 mL glacial acetic acid was added to sodium 1-heptanesulfonate; the volume of both solutions was expanded to 1 L by adding methanol 25%) (Taghizadeh et al. 2021b). L-Ascorbic acid (HPLC grade) was used as standard. The standard was injected via an auto sampler connected to the HPLC system and chromatograms were recorded. Finally, calibration curves were separately constructed by plotting the mean area under the curve against the relevant concentration. The calibration curve was plotted by calculating the ratio of the peak area of standard to the peak area of internal standard against the relevant concentration (Ponder and Hallmann 2020; Taghizadeh et al. 2020a).

Total Protein Content

Total protein content was determined using the slightly modified method that was earlier described by Chalé et al. (2014). Briefly, 1.0 kg of each sample was extracted using 3% sodium bisulfite (1:10 [w:v] ratio, pH 8) for 1 h. In order to separate the fiber solids from the starch-containing liquid portion and protein, the extract was passed through a 0.17-mm mesh size sieve. The residual solids were washed by 3% sodium bisulfite. In order to separate the starch and solubilized protein, the final digested sample was left for 30 min. Then, 1.0 M HCl was used for adjusting the last pH to 4.2. The suspension was assayed through centrifugation (1317 g) and then freeze-dried at –40 °C for 15 min (Chalé et al. 2014; Taghizadeh et al. 2020d).

Dietary Fiber Content

Dietary fiber was mostly quantified as crude fiber according to the Association of Official Agricultural Chemists 2011.25 method. The samples were crushed through a 0.5-mm sieve. Then, 1 g of sample was weighed in an incubation bottle. In order to remove the starch, pancreaticα-amylase amyloglucosidase (AMG) was added to each bottle, and the bottles were then incubated in a water bath (37 °C, for 16 h). The enzyme protease was also added to remove proteins (60 °C, for 30 min). Water-soluble/insoluble polysaccharides and oligosaccharides were analyzed as separate fractions (Samadi et al. 2020). The hydrolyzed sample was filtered. Water-soluble/insoluble polysaccharides were separated, dried, and weighed. The oligosaccharide was hydrolyzed by AMG and analyzed by HPLC after deionization. Sorbitol was used as an internal standard for oligosaccharide analysis. The total dietary fiber amount consisted of water-soluble/insoluble polysaccharides and oligosaccharide. The dietary fiber contents were calculated in fresh weight (grams/100 g) and therefore corrected by moisture. Dried weights were only used for statistical treatment (Pastell et al. 2019).

TSS

TSS was determined according to Koodkaew (2019). A fresh 1-g sample was extracted with 10 mL of distilled water and then centrifuged at 5000 rpm for 10 min. Then, 0.5 mL of the supernatant (sugar solution) was added to 5% phenol (0.5 mL) and H_2SO_4 (2.5 mL). The mixture was incubated for 10 min and then shaken and kept in a water bath (30 °C, for 20 min). The absorbance was read at 490 nm. Glucose was used as the standard for plotting the calibration (Koodkaew 2019).

Element Content

The microwave digestion system (Milestone Ethos Microsynth Oven, Germany) was used for digestion of the nutritive elements (Ca, Fe, Mg, Cu, and Zn). The samples were homogenized with 10 W intervals and 1000 W maximum power. Digestion conditions are reported in Table 3 (Taghizadeh et al. 2021a). Inductively coupled plasma-optical emission spectrometry (ICP-OES) (SPECTRO ARCOS, Germany) was used for simultaneous multi-element detection with Torch type of Flared end EOP Torch 2.5 mm. The plasma power was 1.2 kW, the argon flow rate was 15.0 L/min with an auxiliary flow of 1.50 L/min, the read time was 60 s, and the nebulizer pressure was 250 kPa. Operating optimal parameters were: radio-frequency generator (1400 W). Sample uptake time, rinse time, and initial stabilization time were 240 total, 45 s, and pre-flush 45 s, respectively. Both delay time and time between replicate analyses were zero. Type of detector solid state and spray chamber was CCD, cyclonic, and modified lichte, respectively. Prewash pump speed was 60 rpm (for 15 s) and 30 rpm (for 30 s). The prewash time was 45 s; at the end, sample injection pump speed was 30 rpm. The extraction procedure was performed as follows: 10 g of each sample was digested using 60 mL of HNO_3 and 20 mL of concentrated H_2O_2 (30%) by using microwave digestion system for 25 min, and then diluted to 100 mL using 2% HNO_3. Blank preparation was done in the same way. Finally, clear liquid samples were analyzed by ICP-OES (Taghizadeh et al. 2020b). For the quantitative analysis of metals in samples, multi-element (Ca, Fe, Mg, Cu, and Zn) standard solution at 1000 mg/L, was prepared for plotting calibration curves. Stock solution was diluted with 0.2% HNO_3 solution. For recovery determination, spiked samples were prepared in triplicates and then treated according to the procedure described in sample preparation. The recoveries were calculated using the spiked calibration curves (Taghizadeh et al. 2020c).

ANV

The ANV index depended on the type and amount of the main components in the matrix. In order to compare the genotypes, ANV was calculated by Eq. 2 (Natto et al. 2022).

$$ANV = \frac{Ca\ (mg)}{100} + \frac{Fe\ (mg)}{100} + \frac{Protein\ (g)}{5} + \frac{Vitamin\,C\ (mg)}{40} + Dietary\,fiber\ (g) \quad (2)$$

Biochemical Analysis

TPC

TPC was determined using the Folin-Ciocalteau reagent. The extract (100 µL) and Folin-Ciocalteau reagent (0.5 mL) were mixed and diluted with distilled water (10 times).

Table 3 Operating program used for microwave digestion

Phase	Initial temperature (°C)	Final temperature (°C)	Time (min)	Power (W)
1	25	90	5	700
2	90	90	3	600
3	90	170	10	600
4	170	170	7	600

Then, 7 mL of distilled water was added and kept at room temperature for 5 min. In addition, 1.5 mL of the sodium bicarbonate solution (60 mg/mL) was mixed. The solution was then incubated in a dark place for 2 h. The absorbance was read at 725 nm against the blank by UV-visible spectrophotometer (Cecil, UK). The calibration curve was plotted using a standard solution of gallic acid (GAE) (0.2–1 mg/mL). Results were expressed as milligrams GAE/100 g dried extract (DE) (Taghizadeh et al. 2018).

TFC

TFC content was measured via the colorimetric assay. Briefly, 5 mL of aluminium trichloride ($AlCl_3$) (2%) was mixed with 0.5 mg/mL of the extract. The absorbance was read at 367 nm. TFC was expressed as milligrams quercetin (QUE)/100 g DE (Rahmati et al. 2015; Yaman 2021).

TMAC

In order to determine the TMAC content, 1.0 mL potassium chloride solution (0.2 mol/L) (pH = 1.0) and 1.0 mL sodium acetate buffer (1 mol/L) (pH = 4.5) were added to 2.0 mL of extracts. The absorbance was then measured at 517 and 700 nm. In order to calculate the sample absorbance as well as TMAC content, the following equations were used. The result was expressed as milligrams cyanidin-3-glucoside (cy-3-glu)/100 g DE (Eq. 3 and 4) (Yaman 2022; Yang et al. 2012; Zarei et al. 2011).

$$Sample\,absorbance = Absorbance\,at\,517nm - Absorbance\,at\,700nm \quad (3)$$

$$TMAC\ content(mg/g) = \frac{A}{\varepsilon L \times MW \times 1000 \times Dillution\,Factor} \quad (4)$$

- A: Difference of sample absorbance between both pHs (1.0 and 4.5)
- ε: Molar extinction coefficient for cy-3-glu (26,900)
- MW: Molecular weight of cy-3-glu (449.2 g/mol)

Antioxidant Assay

DPPH To evaluate DPPH scavenging power of the fruit extracts, various volumes of the extract ranging from 0.2 to 4 mL (with 0.2-mL intervals) were added to the falcon tubes (15 mL). The final volumes were adjusted to 4 mL with distilled water. Then, 1 mL of 0.05 mM methanolic DPPH was added to the solution and stored in a dark place. The tubes were vortexed and the mixture was left at room temperature for 1 h. The absorbance was measured in 515 nm and the DPPH scavenging percentage was calculated (Aminifard et al. 2012a). The amount of the DE that is able to scavenge 50% of the DPPH molecules was considered as the half maximal inhibitory concentration (IC_{50}). The inhibition of the DPPH free radical was calculated using the following equation (Taghizadeh et al. 2019):

$$\text{DPPH scavenging effect } (\%) = 1 - \frac{Asample}{Ablank} \times 100 \quad (5)$$

- A_{blank}: The absorbance of the control reaction (including all reagents except the test sample).
- A_{sample}: The absorbance of the test sample.

FRAP Two types of FRAP reagents were used: (i) FRAP reagent (sodium acetate buffer, pH = 3.6 + 10 mM 2,4,6-Tris [2-pyridyl]-s-triazine [TPTZ] solution + $FeCl_3.6H_2O$ [10:1:1 v:v:v]), (ii) Standard FRAP reagent (sodium acetate buffer, pH = 3.6 + 10 mM TPTZ solution + distilled water [10:1:1 v:v:v]). The first and second reagents were added to fruit extract and standard solutions, respectively. The buffer included 46.3 mL of acetic acid (0.2 M) and 3.7 mL of sodium acetate trihydrate (0.2 M), then it was diluted with distilled water. TPTZ was dissolved in 40 mM HCl on a magnetic stirrer heater at 50 °C and kept away from light. A total of 20 µL of the fruit extract was diluted with 980 µL distilled water and 3 mL of FRAP reagent. The mixture was vortexed and incubated in a water bath (37 °C, for 30 min). For the blank, 1 mL of distilled water was added to 3 mL standard FRAP reagent. The spectrophotometer was zeroed using the blank and the sample was read at 593 nm. An aqueous solution of $FeSO_4.7\ H_2O$ (0–75 µM) was used for calibration curve (Taghizadeh et al. 2018).

Color Determination

For all the samples, two readings were taken on reverse sides. Both external and internal color assays were performed. The CIE Lab scale was used to determine the color indices L*, a*, and b* via a colorimeter (Model Konica Minolta Chroma Meters CR-410) (Crecente-Campo et al. 2012).

Statistical Analysis

Statistical analysis at the 5% level ($p \le 0.05$) was carried out by Minitab software version 16 using Tukey's range test for comparing means. All charts were drawn using Microsoft (USA) Excel 2016. The NMDS and PCA were carried out using SPSS version 16 (IBM, USA).

Table 4 Morphological traits of barberry genotypes

Genotype	Berry length (mm)	Berry width (mm)	Pulp (%)	Number of non-aborted seed	Number of aborted seed	100-Berry fresh weight (g)	100-Berry dried weight (g)
Seedless	10.28^{c}	6.41^{abc}	97.37^{a}	0.00^{b}	3.27^{a}	16.78^{c}	3.32^{e}
2-2	9.87^{c}	7.06^{a}	98.17^{a}	1.57^{a}	1.37^{bc}	23.15^{ab}	6.53^{abc}
4-1	10.26^{c}	6.00^{cd}	85.76^{b}	1.57^{a}	1.37^{bc}	21.58^{b}	7.60^{a}
5-1	7.93^{d}	5.33^{def}	71.58^{c}	1.47^{a}	0.33^{de}	11.68^{de}	4.75^{de}
5-2	9.72^{c}	5.70^{cde}	98.50^{a}	0.03^{b}	3.17^{a}	14.26^{cde}	4.54^{de}
5-3	10.57^{bc}	6.03^{cd}	88.19^{b}	1.17^{a}	2.00^{b}	21.85^{b}	6.85^{a}
8-3	11.72^{a}	6.99^{ab}	90.27^{b}	1.23^{a}	1.53^{b}	26.78^{a}	7.82^{a}
10-1	8.32^{d}	4.74^{f}	51.93^{e}	1.73^{a}	0.13^{de}	11.50^{e}	4.94^{cde}
11-1	7.66^{d}	5.11^{ef}	74.08^{c}	1.40^{a}	0.77^{cd}	11.16^{e}	4.04^{de}
12-1	11.31^{ab}	6.01^{cd}	61.78^{d}	1.70^{a}	0.07^{e}	21.01^{b}	6.72^{ab}
13-1	10.66^{bc}	6.16^{bcd}	86.84^{b}	1.33^{a}	1.70^{b}	23.44^{ab}	7.04^{a}
14-2	7.98^{d}	5.72^{cde}	67.66^{cd}	1.67^{a}	0.00^{e}	15.88^{cd}	5.10^{bcd}

In each column, lowercase superscripts (a, b, c, etc.) express statistical variations among different genotypes ($p \leq 0.05$)

Table 5 Nutritional traits of barberry genotypes

Genotype	Ascorbic acid (mg/100 g)	Protein (%)	Crude fiber (%)	TSS (%)
Seedless	61.93^{b}	3.41^{abc}	10.04^{cd}	59.61^{a}
2-2	22.03d^{e}	3.68^{abc}	10.53^{cd}	4.05^{c}
4-1	86.89^{a}	3.12^{bc}	6.23^{d}	3.91^{c}
5-1	25.20^{de}	3.00^{bc}	7.97^{d}	5.34^{c}
5-2	6.35^{e}	2.77^{c}	9.23^{cd}	4.25^{c}
5-3	45.20^{bc}	3.40^{abc}	8.51^{cd}	25.67^{b}
8-3	22.18^{de}	3.44^{abc}	7.24^{d}	3.65^{c}
10-1	45.20^{bc}	3.62^{abc}	54.96^{a}	2.77^{c}
11-1	21.16^{e}	3.06^{bc}	7.40^{d}	4.04^{c}
12-1	41.14^{cd}	4.11^{ab}	20.20^{b}	3.44^{c}
13-1	59.19^{bc}	3.03^{bc}	8.33^{d}	3.23^{c}
14-2	89.00^{a}	4.26^{a}	14.65^{bc}	3.79^{c}

In each column, lowercase superscripts (a, b, c, etc.) express statistical variations among different genotypes ($p \leq 0.05$)
TSS total soluble sugar

Results

Morphological Traits

Significant morphological differences were observed among the barberry genotypes (Table 4). Berry length ranged from 7.66 mm (genotype #11-1) to 11.72 mm (genotype #8-3).

Fig. 2 Aborted seeds (*left*) and non-aborted seeds (*right*)

Genotypes #2-2 and #10-1 exhibited the maximum and minimum berry width (7.06 and 4.74 mm, respectively). The pulp percentages were in the range of 51.93–98.5% (Table 4). The lowest fresh and dried weights of 100 berries among all studied samples were found in genotypes #11-1 (11.16 g) and seedless (3.32 g), respectively (Table 4, Fig. 2). Genotype #10-1 showed the highest non-aborted seed number (1.73 seed/berry). The average number of aborted seeds varied from zero in #14-2 to 3.27 in the seedless genotype. In addition, genotype #5-2 had a high value of aborted seeds (3.17) (Table 4).

Nutritional Traits

Results indicated that among the investigated genotypes, #14-2 and #4-1 showed the highest levels of ascorbic acid (89.00 and 86.89 mg/100 g DE, respectively). The lowest

Table 6 Element contents of barberry genotypes

Genotype	Ca (ppm)	Cu (ppm)	Fe (ppm)	Mg (ppm)	Zn (ppm)	ANV
Seedless	654.98^{c}	5.08^{bc}	116.60$^{a–d}$	324.13^{c}	13.65^{ab}	18.76^{c}
2-2	754.23^{c}	4.22^{c}	85.80^{de}	392.34^{c}	11.58^{ab}	16.86^{c}
4-1	668.41^{c}	4.09^{c}	81.01^{e}	316.84^{c}	12.42^{ab}	13.75^{c}
5-1	1012.17^{c}	5.05^{bc}	95.39^{de}	452.23^{c}	8.13^{b}	14.99^{c}
5-2	1097.62^{c}	4.23^{c}	129.65^{abc}	403.84^{c}	12.26^{ab}	17.52^{c}
5-3	1000.48^{c}	4.09^{c}	96.85^{cde}	397.42^{c}	9.80^{b}	16.16^{c}
8-3	782.23^{c}	4.20^{c}	130.84^{ab}	395.27^{c}	10.51^{ab}	15.80^{c}
10-1	3691.62^{a}	4.82^{bc}	138.49^{a}	1426.39^{a}	14.91^{ab}	67.43^{a}
11-1	1033.43^{c}	5.25^{b}	96.56^{cde}	463.55^{c}	18.85^{a}	14.40^{c}
12-1	1770.48^{b}	4.70^{bc}	96.16^{cde}	887.56^{b}	11.28^{ab}	28.63^{b}
13-1	862.69^{c}	4.30^{bc}	97.65$^{b–e}$	346.96^{c}	13.51^{ab}	16.16^{c}
14-2	1107.25^{c}	6.80^{a}	131.98^{a}	461.82^{c}	16.82^{ab}	25.43^{b}

In each column, lowercase superscripts (a, b, c, etc.) express statistical variations among different genotypes ($p \leq 0.05$)
ANV average nutritive value

Table 7 Biochemical traits of barberry genotypes

Genotype	Juice (%)	Moisture (%)	TPC (mg/100 g DE)	TFC (mg/100 g DE)	TMAC (mg/100 g DE)	IC_{50} (mg)	FRAP (µM $Fe2^{+}$)
Seedless	71.16^{a}	9.67^{abc}	1273.22^{abc}	492.56^{c}	343.71^{c}	44.98^{c}	170.32^{a}
2-2	70.46^{a}	9.33^{abc}	1274.00^{abc}	276.15^{def}	35.96^{g}	86.83^{ab}	133.39^{ab}
4-1	54.77^{de}	10.67^{abc}	1386.72^{a}	273.50^{ef}	88.81^{efg}	76.84^{ab}	109.29^{bc}
5-1	56.43^{cde}	11.00^{abc}	1482.61^{a}	682.59^{b}	371.94^{bc}	30.90^{cde}	132.18^{ab}
5-2	64.38^{b}	11.00^{abc}	1225.72^{abc}	342.13^{def}	98.02^{ef}	103.23^{a}	136.69^{ab}
5-3	60.67^{bc}	8.67^{bc}	1342.76^{ab}	249.43^{f}	123.05^{e}	76.05^{b}	136.01^{ab}
8-3	71.87^{a}	11.33^{ab}	1266.43^{abc}	352.68$^{c–f}$	105.10^{ef}	82.56^{ab}	129.86^{ab}
10-1	51.22^{e}	10.33^{abc}	1195.44^{abc}	415.92^{cde}	245.86^{d}	16.74^{de}	157.86^{ab}
11-1	53.92^{de}	11.33^{ab}	1200.43^{abc}	652.31^{b}	408.29^{ab}	38.72^{cd}	111.16^{bc}
12-1	64.07^{b}	8.33^{c}	756.30^{d}	418.46^{cd}	127.29^{e}	22.69^{cde}	72.20^{c}
13-1	58.11^{cd}	11.00^{abc}	1011.42^{bcd}	219.72^{f}	52.68^{fg}	80.19^{ab}	118.37^{abc}
14-2	64.28^{b}	12.00^{a}	965.31^{cd}	837.53^{a}	452.06^{a}	4.68^{e}	103.43^{bc}

In each column, lowercase superscripts (a, b, c, etc.) express statistical variations among different genotypes ($p \leq 0.05$)
TPC total phenolic content, *DE* dried extract, *TFC* total flavonoid content, *TMAC* total monomeric anthocyanin content, IC_{50} half maximal inhibitory concentration, *FRAP* ferric reducing antioxidant power

value was related to genotype #5-2 (6.35 mg 100 mg/100 g DE). Moreover, the highest amount of protein (4.26%) was observed in #14-2, followed by genotypes #12-1 and #2-2 with 4.11% and 3.68%, respectively. Genotype #5-2 had the lowest protein content (2.77%) (Table 5). As shown in Table 5, the crude fiber content range was between 6.23% and 54.96% in genotypes #4-1 and #10-1, respectively. Also, 12 genotypes showed significant differences in terms of TSS percent. The potential genotypes for TSS were seedless (59.61%) and #5-1 (25.67%), respectively (Table 5). Element contents are given in Table 6. Significant differences between mean values of nutritive elements were determined. Genotype #10-1 showed the most ANV value (67.43) with the highest levels of Fe (138.49 ppm) and Ca (3691.62 ppm) (Table 6).

Biochemical Traits

The percentage of fruit juice ranged from 51.22% (#10-1) to 71.87% (#8-3), and the moisture content ranged from 8.33–12%. As shown in Table 7, genotype #5-1 showed the highest amount of TPC (1482.61 mg GAE/100 g DE). The TFC level also ranged from 219.72–837.53 mg QUE/100 g DE. Moreover, the highest level of TMAC was related to genotype #14-2 (452.06 mg cy-3-glu/100 g DE). The IC_{50} value of the #14-2 (4.68 µg/mL) was significantly lower than other genotypes (Table 7). The analytical performance for plotting the calibration curve in the FRAP assay is shown in Table 8. In this assay, the seedless genotype developed the bluest color which was detectable through spectrophotometry and showed the highest antioxidant activity by 170.32 µM $Fe2^{+}$, whereas the genotype #12-1 showed the lowest antioxidant activity (72.20 µM Fe2+). Accordingly,

Table 8 Analytical performance for plotting the calibration curve in the FRAP assay

	Concentrations (µM)							
Standard ingredients	0 (Blank)	3.75	7.50	15.00	30.00	45.00	60.00	75.00
Distilled water (µL)	1000.00	985.00	970.00	940.00	880.00	820.00	760.00	700.00
Standard FRAP reagent (mL)	3.00	3.00	3.00	3.00	3.00	3.00	3.00	3.00
Standard solution (µL)	0.00	15.00	30.00	60.00	120.00	180.00	240.00	300.00

Table 9 Color traits of barberry genotypes

Genotype	Color indices of fresh fruit			Color indices of dried fruit		
	L*	a*	b*	L*	a*	b*
Seedless	30.20^{bc}	30.62^{b}	9.14^{cd}	28.87^{abc}	15.76^{b}	3.65^{d}
2-2	38.13^{a}	35.70^{a}	21.52^{a}	31.42^{a}	17.57^{ab}	7.71^{a}
4-1	27.45^{bcd}	25.98^{c}	7.68^{e}	26.97^{bc}	16.10^{b}	4.21^{cd}
5-1	22.83^{e}	16.78^{d}	2.11^{f}	28.94^{abc}	7.97^{c}	0.08^{ef}
5-2	30.84^{bc}	27.56^{bc}	9.53^{c}	27.62^{bc}	17.33^{ab}	5.21^{bcd}
5-3	30.47^{bc}	26.95^{c}	8.22^{de}	27.14^{bc}	17.28^{b}	5.45^{bc}
8-3	41.29^{a}	37.76^{a}	17.97^{b}	29.58^{ab}	20.22^{a}	6.87^{ab}
10-1	27.63^{bcd}	6.51^{e}	-1.58^{g}	29.57^{ab}	3.75^{d}	-0.72^{fg}
11-1	23.34^{de}	19.29^{d}	2.74^{f}	27.33^{bc}	10.28^{c}	1.20^{e}
12-1	26.71^{cde}	4.46^{ef}	-2.12^{gh}	26.37^{c}	2.75^{d}	-0.58^{f}
13-1	31.39^{b}	24.93^{c}	7.04^{e}	28.55^{abc}	17.91^{ab}	6.56^{ab}
14-2	29.44^{bc}	2.31^{f}	-3.23^{h}	31.30^{a}	1.06^{d}	-2.34^{g}

In each column, lowercase superscripts (a, b, c, etc.) express statistical variations among different genotypes ($p \leq 0.05$)
*L** lightness, *a** greenness [–] to redness [+], *b** blueness [–] to yellowness [+]

antioxidant activity of the genotypes had a linear correlation with their major constituents (Table 7).

Color Traits

Color indices of fresh and dried fruits are shown in Table 9. In fresh and dried fruits, the L* values were in the range of 22.83–41.29 and 26.37–31.42, respectively; the a* values varied from 2.31–37.76 and 1.06–20.22, respectively; b* values ranged from –3.23 to 21.52 and –2.34 to 7.71, respectively. Genotype #14-2 with a unique black color and a bluish wax showed 29.44, 2.31, and –3.23 for L*, a*, and b* color indices, respectively (Table 9).

NMDS and PCA

The NMDS computes the two-dimensional locus for each individual variable including TPC, TFC, and TMAC. In terms of TPC, the results of NMDS ordination showed the separation of genotype #12-1 (Fig. 3). The TFC and TMAC results showed the separation of genotype #14-2 (Figs. 4 and 5). Final coordinates of NMDS for 12 selected barberry genotypes are presented in Table 10. The stress values for TPC, TFC, and TMAC were low, which indicted the high goodness of fit of the ordination distances to the observed distances (Table 11). The other pattern was obtained among the constructed dendrogram for cluster anal-

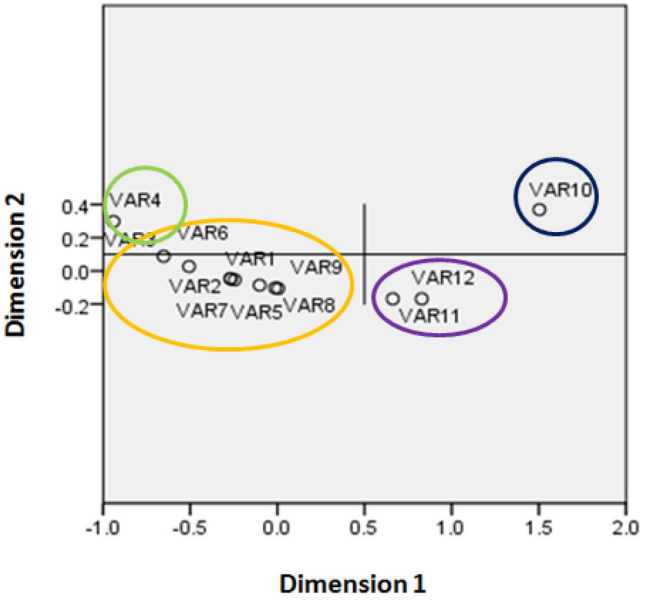

Fig. 3 Non-metric multidimensional scaling for total phenolic compound of 12 selected barberry genotypes. *VAR1* seedless, *VAR2* genotype 2-2, *VAR3* genotype 4-1, *VAR4* genotype 5-1, *VAR5* genotype 5-2, *VAR6* genotype 5-3, *VAR7* genotype 8-3, *VAR8* genotype 10-1, *VAR9* genotype 11-1, *VAR10* genotype 12-1, *VAR11* genotype 13-1, *VAR12* 14-2

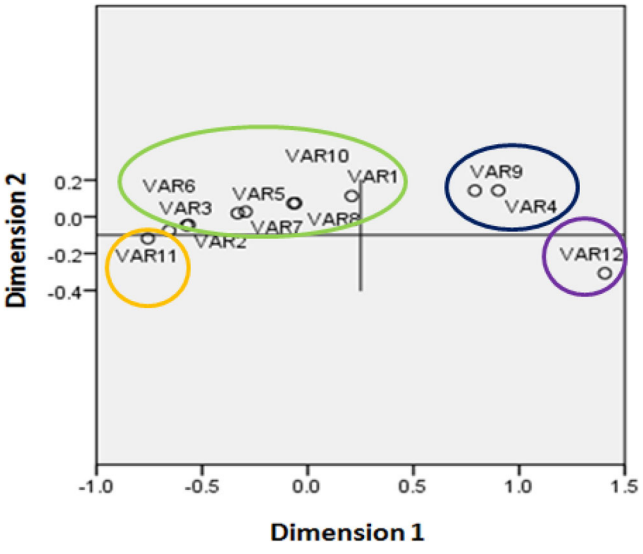

Fig. 4 Non-metric multidimensional scaling for total flavonoid compound of 12 selected barberry genotypes. *VAR1* seedless, *VAR2* genotype 2-2, *VAR3* genotype 4-1, *VAR4* genotype 5-1, *VAR5* genotype 5-2, *VAR6* genotype 5-3, *VAR7* genotype 8-3, *VAR8* genotype 10-1, *VAR9* genotype 11-1, *VAR10* genotype 12-1, *VAR11* genotype 13-1, *VAR12* 14-2

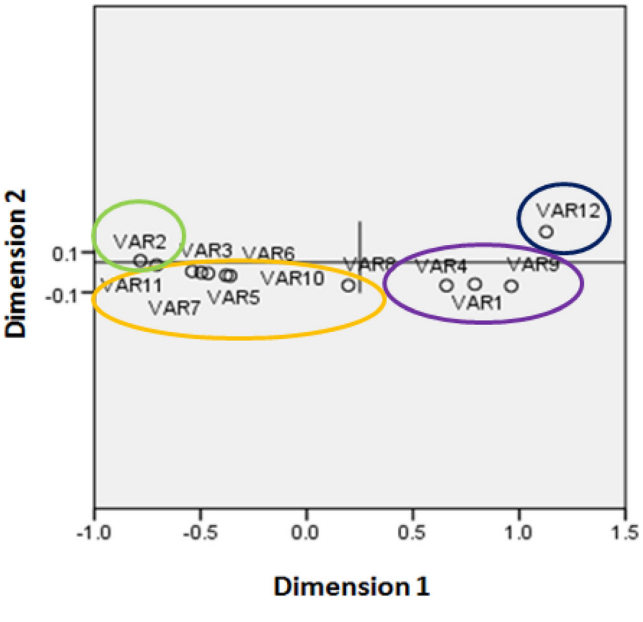

Fig. 5 Non-metric multidimensional scaling for total monomeric anthocyanin content of 12 selected barberry genotypes. *VAR1* seedless, *VAR2* genotype 2-2, *VAR3* genotype 4-1, *VAR4* genotype 5-1, *VAR5* genotype 5-2, *VAR6* genotype 5-3, *VAR7* genotype 8-3, *VAR8* genotype 10-1, *VAR9* genotype 11-1, *VAR10* genotype 12-1, *VAR11* genotype 13-1, *VAR12* 14-2

ysis and bidimensional biplots for PCA. As described in Fig. 6, the dendrograms made it possible to separate different genotypes into the main groups, each representing a distinct chemotype. Seedless and genotype #5-2 exhibited a distinct separation compared with the other genotypes (Group I). The second group (II) comprised two genotypes (#2-2 and #8-3). Group III was made up of three genotypes including #4-1, #13-1, and #5-3. Genotypes #5-1, #11-1, and #14-2 were classified in group VI, as well as genotypes #10-1, #12-1 were regarded in group V (Fig. 6).

The correlation coefficient between the two variables showed the intensity of the relationship between the two variables (from +1 to –1). Lack of correlation was not a reason for lack of relationship. Therefore, in some cases where measuring an attribute was costly, complex, time–consuming, and difficult, other attributes that have high significant correlations with this attribute could be used. Correlation coefficients between traits were measured in two separate groups (morphological-nutritional traits and biochemical traits) in the studied genotypes, which are given in Tables 12 and 13. Simple correlation coefficients between traits showed that there was a significant correlation between some of the measured traits (Tables 12 and 13).

Discussion

In this study, we analyzed several morphological, nutritional, and biochemical traits of 12 *Berberis* genotypes and assessed their correlation based on mathematical models. On the basis of our results, significant morphological, nutritional, and biochemical differences were observed among the barberry genotypes. Alizade et al. (2020) studied 15 different barberry genotypes from Iran. Their results showed that the morphological traits were significantly different. In this regard, #Shirvan 3 showed the highest amount of 100 berries DW (8.55 g). In terms of the number of leaves per cluster (18 leaves per cluster) and the length of fruit tail (9.6 mm), Birjand seedless genotypes showed the highest average value. Among their selected genotypes, #Golestan 5 showed the most length and diameter of fruits (11.97 mm and 1.98 mm, respectively) (Alizade et al. 2020). The previous study reported that the variation among the fresh and dried weights of barberry fruit could depend on the genotype and environmental conditions (Zarei et al. 2010). According to the findings of a study on barberry samples in Iran, different genotypes were expected to represent different fresh and dried weights of 100 berry fruits (3.69 and 1.46 g, respectively) (Khayyat et al. 2018). Yildiz et al. reported that one-berry weight of *B. vulgaris* ranged from 0.08–0.36 g (Yildiz et al. 2014). Fruit set and seed set are important factors in breeding programs. Obviously, seedless fruits are desirable for consumers. Ebadi et al. (2010) reported that seedless barberry produced 20% seedy fruits in pollination with wild barberries (Ebadi et al. 2010). Our results confirmed the findings of the relevant study.

Table 10 Final coordinates of non-metric multidimensional scaling for 12 selected barberry genotypes

Genotype	TPC		TFC		TMAC	
	Dimension		Dimension		Dimension	
	1	2	1	2	1	2
Seedless	–0.268	–0.048	0.208	0.113	0.657	–0.066
2-2	–0.271	–0.047	–0.564	–0.044	–0.784	0.058
4-1	–0.653	0.088	–0.573	–0.047	–0.540	0.004
5-1	–0.941	0.298	0.901	0.144	0.791	–0.059
5-2	–0.102	–0.086	–0.332	0.019	–0.497	–0.001
5-3	–0.507	0.026	–0.656	–0.076	–0.380	–0.015
8-3	–0.244	–0.054	–0.294	0.028	–0.464	–0.006
10-1	–0.005	–0.107	–0.069	0.073	0.195	–0.065
11-1	–0.013	–0.103	0.791	0.143	0.962	–0.069
12-1	1.503	0.369	–0.060	0.075	–0.360	–0.018
13-1	0.662	–0.169	–0.757	–0.118	–0.707	0.036
14-2	0.828	–0.169	1.405	–0.308	1.127	0.201

TPC total phenolic content, *TFC* total flavonoid content, *TMAC* total monomeric anthocyanin content

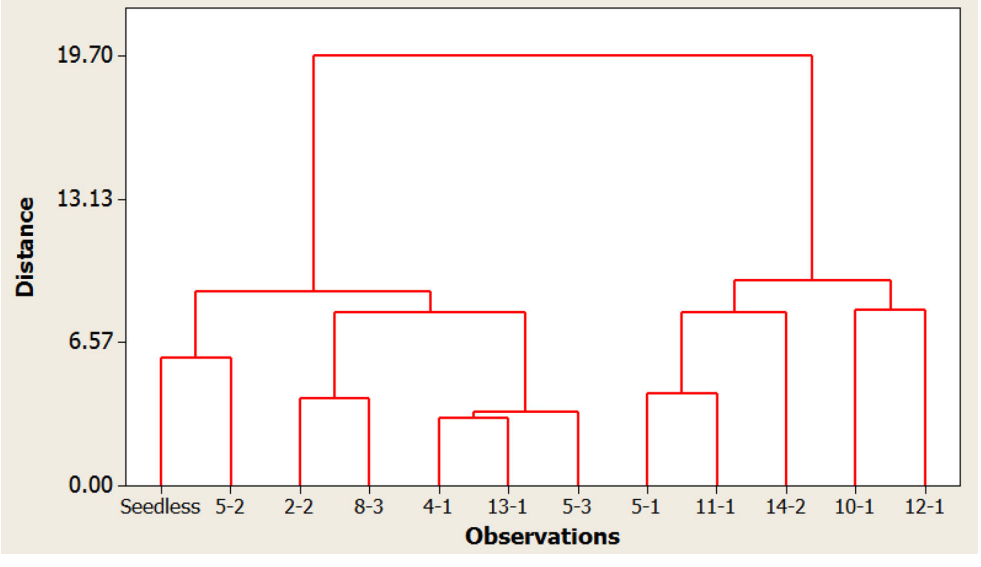

Fig. 6 Average-linkage dendrogram of the barberry genotypes resulting from the cluster analysis

The seed percentage observed in the seedless genotype (2.63%) was due to open pollination. In a previous study, there was a strong negative correlation (r = –1) between pulp and seed percentages. Moreover, this negative correlation (r = –0.91) was obtained between the number of non-aborted and aborted seeds. Parthenocarpy or stenospermocarpy might have increased the pulp percentage. The fruit color could be an appropriate representative of its quality and maturity state and might have affected consumer acceptability (Tural and Koca 2008; Yildiz et al. 2014).

Similar results were reported from Iran. The values of L*, a*, and b* indices in *B. vulgaris* were 20.82, 34.84 and, 18.91, respectively (Ardestani et al. 2013). The other reports also showed the various colors of barberry genotypes (Akbulut et al. 2009; Yildiz et al. 2014). Genotype #14-2, with a distinctive black color and a bluish wax, was similar to the "Late blue" blueberry cultivar (L* = 26.16, a* = –0.32, and b* = –3.88) (Saftner et al. 2008). This color variety of barberry genotypes can provide an opportunity for food industries as natural and safe food colors in dairy, candies, jellies, and etc. Furthermore, barberry color is used to dye natural silk (Pruthi et al. 2008).

Considering the use of fresh and dry barberry fruits, fruit juice, and moisture index, are the other important factors (Ardestani et al. 2013; Zarei et al. 2010). Different types of consumption and processing are determined by considering the content of fruit juice, while different methods of packaging and storage are determined by the moisture content of dried fruits. Juicy genotypes can be used for fresh juice, while genotypes with less moisture content can potentially be more useful for edible powders in yogurt and herbal tea (Wallace and Giusti 2008).

Table 11 Goodness of fit of non-metric multidimensional scaling for 12 selected barberry genotypes

Output parameters	Value		
	TPC	TFC	TMAC
Normalized raw stress	0.00076	0.00053	0.00032
Stress-I	0.02760[a]	0.02308[c]	0.01781[e]
Stress-II	0.04580[a]	0.03937[c]	0.03064[e]
S-Stress	0.0245[b]	0.00105[d]	0.00040[f]
Dispersion accounted for (D.A.F)	0.99924	0.99947	0.99968
Tucker's coefficient of congruence	0.99962	0.99973	0.99984

TPC Total phenolic content, *TFC* Total flavonoid content, *TMAC* Total monomeric anthocyanin content
[a]Optimal scaling factor = 1.001
[b]Optimal scaling factor = 1.014
[c]Optimal scaling factor = 1.001
[d]Optimal scaling factor = 1.009
[e]Optimal scaling factor = 1.000
[f]Optimal scaling factor = 1.003

Akbulut et al. (2009) determined the moisture of fresh *B. vulgaris* (71.42%) (Akbulut et al. 2009). Based on our results, the seedless genotype showed the same value. Phenolic compounds are a crucial portion of the daily human diet and are well known for their antioxidant properties (Fattahi et al. 2021). Gallic acid, *p*-coumaric acid, chlorogenic acid, vanillic acid, caffeic acid, syringic acid, trans-ferulic acid, sinapic acid, quercetin, rutin, apigenin, and cinnamic acid are the main phenolic compounds of barberry fruit (Eroğlu et al. 2020; Gholizadeh-Moghadam et al. 2019). Similar results were found in *B. vulgaris*, where TPC ranged from 261.68 to 623.07 mg GAE/100 g FW (Hassanpour and Alizadeh 2016). Eroglu et al. determined the TPC in wild *B. vulgaris* and *B. crataegina* (148 and 448.3 µg GAE/100 g DW, respectively) (Eroğlu et al. 2020). In another study performed in Turkey, it was found that TPC ranged from 3590–2532 mg GAE/L (Yildiz et al. 2014). Ardestani et al. showed that TPC was 48,000 and 28,000 mg GAE/100 g DE in *B. integerrima* and *B. vulgaris*, respectively (Ardestani et al. 2013). It is known that genotype and environmental factors of various zones affect the quality of the produced fruits (Ardestani et al. 2013; Chizzola et al. 2014; Yildiz et al. 2014). TFC is one of the most abundant secondary metabolites in barberry fruits. Hassanpour and Alizadeh (2016) reported the range of 132.66–280.0 mg catechin/100 g FW for different barberry genotypes (Hassanpour and Alizadeh 2016). The previous study showed that the concentrations of flavonoid and flavonol in *B. vulgaris* were 12.2 and 25.3 mg/g, respectively (Rahimi-Madiseh et al. 2017). A previous study determined the levels of TMAC in blueberry extract (7 g monomeric anthocyanin/100 g DW) (Jiménez-Aguilar et al. 2011). In another study, TMAC ranged from 16.32 to

Table 12 Correlation of morphological and nutritional traits

No.	Trait	1	2	3	4	5	6	7	8	9	10	11	12	13
1	Juice percentage	1	–	–	–	–	–	–	–	–	–	–	–	–
2	Moisture percentage	0.054^{ns}	1	–	–	–	–	–	–	–	–	–	–	–
3	Pulp percentage	0.594^{ns}	–0.148^{ns}	1	–	–	–	–	–	–	–	–	–	–
4	Seed percentage	–0.594^{ns}	0.148^{ns}	–1.000*	1	–	–	–	–	–	–	–	–	–
5	Cluster length	0.005^{ns}	0.159^{ns}	0.675**	–0.675**	1	–	–	–	–	–	–	–	–
6	Berry in cluster	0.085^{ns}	0.214^{ns}	0.607^{ns}	–0.607^{ns}	0.606^{ns}	1	–	–	–	–	–	–	–
7	Berry length	0.441^{ns}	0.167^{ns}	0.408^{ns}	–0.408^{ns}	0.546^{ns}	–0.004^{ns}	1	–	–	–	–	–	–
8	Berry width	0.798*	0.278^{ns}	0.710**	–0.710**	–0.489^{ns}	–0.348^{ns}	–0.666**	1	–	–	–	–	–
9	Number of non-aborted seed	–0.482^{ns}	0.512^{ns}	–0.671**	–0.671**	–0.183^{ns}	–0.154^{ns}	–0.231^{ns}	0.198^{ns}	1	–	–	–	–
10	Number of aborted seed	0.484^{ns}	–0.465^{ns}	0.865*	–0.865*	0.506^{ns}	0.312^{ns}	0.422^{ns}	0.433^{ns}	–0.916*	1	–	–	–
11	100-Berry fresh weight	0.413^{ns}	0.381^{ns}	0.416^{ns}	–0.416^{ns}	0.628^{ns}	0.195^{ns}	0.811*	0.826*	0.144^{ns}	0.168^{ns}	1	–	–
12	100-Berry dried weight	–0.130^{ns}	0.431^{ns}	–0.006^{ns}	0.006^{ns}	0.556^{ns}	0.140^{ns}	0.546^{ns}	0.385^{ns}	0.561^{ns}	–0.265^{ns}	0.794*	1	–
13	Volume of dried weights of 100 berry fruits	0.855*	–0.095^{ns}	0.671**	–0.671**	0.214^{ns}	0.047^{ns}	0.602^{ns}	0.783**	–0.609^{ns}	0.687**	0.474^{ns}	–0.118^{ns}	1

ns Not significant
*$p \leq 0.05$, **$p \leq 0.01$

Table 13 Correlation of biochemical traits

No.	Trait	1	2	3	4	5	6	7	8	9	10	11	12	13
1	TPC	1	–	–	–	–	–	–	–	–	–	–	–	–
2	TFC	–0.026^{ns}	1	–	–	–	–	–	–	–	–	–	–	–
3	TMAC	0.099^{ns}	0.940*	1	–	–	–	–	–	–	–	–	–	–
4	Ascorbic acid	0.170^{ns}	0.918*	0.791*	1	–	–	–	–	–	–	–	–	–
5	IC_{50}	0.313^{ns}	–0.581^{ns}	–0.599^{ns}	–0.550^{ns}	1	–	–	–	–	–	–	–	–
6	L of fresh fruit	–0.096^{ns}	–0.532^{ns}	–0.576^{ns}	–0.334^{ns}	0.806*	1	–	–	–	–	–	–	–
7	a of fresh fruit	0.555^{ns}	–0.563^{ns}	–0.460^{ns}	–0.574^{ns}	0.764*	0.525^{ns}	1	–	–	–	–	–	–
8	b of fresh fruits	0.430^{ns}	–0.590^{ns}	–0.566^{ns}	–0.547^{ns}	0.935*	0.742^{ns}	0.927*	1	–	–	–	–	–
9	L of dried fruit	0.114^{ns}	0.261^{ns}	0.216^{ns}	0.381^{ns}	0.322^{ns}	0.545^{ns}	0.022^{ns}	0.242^{ns}	1	–	–	–	–
10	a of dried fruit	0.451^{ns}	–0.673**	–0.570^{ns}	–0.632**	0.650**	0.501^{ns}	0.961*	0.851*	–0.103^{ns}	1	–	–	–
11	b of dried fruit	0.292^{ns}	–0.775*	–0.724**	–0.685**	0.772*	0.683**	0.918*	0.910*	0.016^{ns}	0.955*	1	–	–
12	pH	0.169^{ns}	–0.301^{ns}	–0.250^{ns}	–0.247^{ns}	–0.222^{ns}	–0.167^{ns}	0.300^{ns}	0.062^{ns}	0.401^{ns}	–0.507^{ns}	0.395^{ns}	1	–
13	TSS	0.029^{ns}	0.809*	0.766*	0.674**	–0.481^{ns}	–0.457^{ns}	–0.661^{ns}	–0.605^{ns}	0.280^{ns}	–0.766*	–0.821*	–0.405^{ns}	1

ns Not significant, *TPC* total phenolic content, *TFC* total flavonoid content, *TMAC* total monomeric anthocyanin content, IC_{50} half maximal inhibitory concentration, *L* lightness, *a* greenness [–] to redness [+], *b* blueness [–] to yellowness [+], *TSS* total soluble sugar

*$p \leq 0.05$, **$p \leq 0.01$

91.66 mg/100 g FW for *B. vulgaris* and *B. integerrima*, respectively (Hassanpour and Alizadeh 2016). Ardestani et al. (2013) reported that the anthocyanin content of aqueous extracts of *B. integerrima* and *B. vulgaris* were 812.033 and 62.103 g/100 g DE, respectively (Ardestani et al. 2013).

Our results showed that genotype #10-1 with the lowest pulp percentage had the highest concentration of Ca, Fe, and Mg, while genotype #4-1 showed the lowest concentration of Cu, Fe, and Mg. Genotype #14-2 had the highest level of Cu. The seedless genotype showed the lowest concentration of Ca. Our results were in accordance with those reported by Akbulut et al. (2009). They showed that Ca, Cu, Fe, Mg, and Zn levels of *B. vulgaris* were 2744.06, 4.75, 323.86, 1193.30, and 7.95 ppm, respectively (Akbulut et al. 2009). Hanachi and Golkho (2009) showed that the level of ascorbic acid in *B. vulgaris* was 11,102.81 µg/100 g DW (Hanachi and Golkho 2009). Akbulut et al. (2009) reported the crude cellulose content of wild *B. vulgaris* (9.42%). In another study, the crude fiber content for three barberry species including *B. calliobotrys, B. orthobotrys*, and *B. pseudumbellata* were 0.73, 0.78, and 0.94%, respectively (Awan et al. 2014). TSC, calculated in 14 barberry genotypes, ranged from 15.40 to 18.11 g/100 mL (Ersoy et al. 2018). Barberry fruits are rich in certain organic acids and provide a good source of taste and flavor (Özgen et al. 2012). Accordingly, several studies showed that organic acids including malic, citric, and tartaric acids can cause desirable health effects such as help maintain the immune system, prevent chronic kidney diseases, and reduce the toxic effects of pollutants (Penniston et al. 2007).

Based on antioxidant activity in the current DPPH method, genotype #14-2 had the greatest antioxidant potential. However, the seedless genotype showed the highest antioxidant capacity at 170.32 µM Fe^{2+}. Several studies approved the positive correlation (r = 0.62) between antioxidant activity and phenol contents (Hassanpour and Alizadeh 2016; Ruiz et al. 2010; Sellappan et al. 2002; Tehranifar et al. 2010; Zarei et al. 2010). Barberry is rich in certain nutrients and provides a good source for pharmaceutical and food industries due to its high alkaloids (berberine, berbamine, and palmatine), polyphenols, anthocyanins, flavonoids, caffeic acid, and chlorogenic acid. Most of the biochemical compounds in barberry are easily absorbed by the human body. The barberry fruit is also a good source of fiber, fructose, glucose, minerals, and vitamins (Sarraf et al. 2019).

Based on NMDS analysis, biochemical categories of the compounds found in 12 barberry genotypes showed component variation. In terms of TPC, the results of NMDS ordination showed the separation of genotype #12-1, whereas the TFC and TMAC results showed the separation of genotype #14-2. The low stress values for biochemical compo-

nents indicated the high goodness of fit of the ordination distances to the observed distances.

Conclusion

In this work, morphological, nutritional, and biochemical traits of 12 barberry genotypes were assessed. Moreover, the barberry genotypes were classified by multivariate analysis. NMDS results determined distinct groups of samples, corresponding to TPC, TFC, and TMAC. Various environmental conditions, agricultural practices, and genetic diversity displayed a notable role in biotechnology breeding programs. It seems that variations among species with different environmental parameters considerably affect the level and chemical composition of natural components in plants. To optimize the pharmaceutical and food industry applications of plants, the proper genotypes need to be introduced. It is worth mentioning that seeds of different genotypes exhibited diversity in size, shape, color, and micro-morphology, which needs more investigation in the future.

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Conflict of interest M. Khodabandeh, M. Azizi, A. Balandari, and S.F. Taghizadeh declare that they have no competing interests.

References

Akbulut M, Çalisir S, Marakoglu T, Coklar H (2009) Some physicomechanical and nutritional properties of barberry (Berberis vulgaris L.) fruits. J Food Process Eng 32:497–511

Alemardan A, Asadi W, Rezaei M, Tabrizi L, Mohammadi S (2013) Cultivation of Iranian seedless barberry (Berberis integerrima 'Bidaneh'): a medicinal shrub. Ind Crops Prod 50:276–287

Alizade M, Talebi S, Ramezanpour SS, Ghasemnejad A (2020) Study of morphological characteristics of different Berberis. Spp Genotypes Northeast Iran J Plant Prod Res 27:75–91

Aminifard M, Aroiee H, Azizi M, Nemati H, Jaafar HZ (2012a) Effect of humic acid on antioxidant activities and fruit quality of hot pepper (Capsicum annuum L.). J Herbs Spices Med Plants 18:360–369

Aminifard M, Aroiee H, Nemati H, Azizi M, Khayyat M (2012b) Effect of nitrogen fertilizer on vegetative and reproductive growth of pepper plants under field conditions. J Plant Nutr 35:235–242

Ardestani SB, Sahari MA, Barzegar M, Abbasi S (2013) Some physicochemical properties of Iranian native barberry fruits (abi and poloei): Berberis integerrima and Berberis vulgaris. J Food Pharm Sci 1(3):60–67. https://doi.org/10.14499/jfps

Awan MS, Ali S, Ali A, Hussain A, Ali M (2014) A comparative study of barberry fruits in terms of its nutritive and medicinal contents from CKNP region, Gilgit-Baltistan. Pak J Biodivers Environ Sci 5:9–17

Chalé FGH, Ruiz JCR, Fernández JJA, Ancona DAB, Campos MRS (2014) ACE inhibitory, hypotensive and antioxidant peptide fractions from Mucuna pruriens proteins. Process Biochem 49:1691–1698

Chizzola R, Saeidnejad AH, Azizi M, Oroojalian F, Mardani H (2014) Bunium persicum: variability in essential oil and antioxidants activity of fruits from different Iranian wild populations. Genet Resour Crop Evol 61:1621–1631

Christenhusz MJ, Byng JW (2016) The number of known plants species in the world and its annual increase. Phytotaxa 261: 201–217

Crecente-Campo J, Nunes-Damaceno M, Romero-Rodríguez M, Vázquez-Odériz M (2012) Color, anthocyanin pigment, ascorbic acid and total phenolic compound determination in organic versus conventional strawberries (Fragaria× ananassa Duch, cv Selva). J Food Compos Anal 28:23–30

Ebadi A, Rezaei M, Fatahi R (2010) Mechanism of seedlessness in Iranian seedless barberry (Berberis vulgaris L. var. asperma). Sci Hortic 125:486–493

Eroğlu AY, Çakır Ö, Sağdıç M, Dertli E (2020) Bioactive Characteristics of Wild Berberis vulgaris and Berberis crataegina. Fruits J Chem. https://doi.org/10.1155/2020/8908301

Ersoy N, Muhammed K, Sagbas HI, Ercisli S (2018) Physicochemical diversity among barberry (Berberis vulgaris L.) fruits from Eastern Anatolia. Not Bot Horti Agrobot 46:336–342

Farhadi F, Iranshahi M, Taghizadeh SF, Asili J (2020) Volatile sulfur compounds: the possible metabolite pattern to identify the sources and types of asafoetida by headspace GC/MS analysis. Ind Crops Prod 155:112827

Fatehi M, Saleh TM, Fatehi-Hassanabad Z, Farrokhfal K, Jafarzadeh M, Davodi S (2005) A pharmacological study on Berberis vulgaris fruit extract. J Ethnopharmacol 102:46–52

Fattahi A et al (2021) UPLC-PDA-ESI-QTOF-MS/MS and GC-MS analysis of Iranian Dracocephalum moldavica L. Food Sci Nutr 9:4278–4286. https://doi.org/10.1002/fsn3.2396

Gholizadeh-Moghadam N, Hosseini B, Alirezalu A (2019) Classification of barberry genotypes by multivariate analysis of biochemical constituents and HPLC profiles. Phytochem Anal 30:385–394

Hanachi P, Golkho S (2009) Using HPLC to determination the composition and antioxidant activity of Berberis vulgaris. Eur J Sci Res 29:47–54

Hassanpour H, Alizadeh S (2016) Evaluation of phenolic compound, antioxidant activities and antioxidant enzymes of barberry genotypes in Iran. Sci Hortic 200:125–130

Imenshahidi M, Hosseinzadeh H (2019) Berberine and barberry (Berberis vulgaris): A clinical review. Phytother Res 33:504–523

Jiménez-Aguilar D, Ortega-Regules A, Lozada-Ramírez J, Pérez-Pérez M, Vernon-Carter E, Welti-Chanes J (2011) Color and chemical stability of spray-dried blueberry extract using mesquite gum as wall material. J Food Compos Anal 24:889–894

Khayyat M, Barati Z, Aminifard MH, Samadzadeh A (2018) Changes in fruit maturity indices and growth pattern along the harvest season in seedless barberry under different altitude conditions. J Berry Res 8:25–40

Koodkaew I (2019) NaCl and glucose improve health-promoting properties in mung bean sprouts. Sci Hortic 247:235–241

Kulkarni SK, Dhir A (2008) On the mechanism of antidepressant-like action of berberine chloride. Eur J Pharmacol 589:163–172

Mohammadi S, Azizi M, Vaezi J, Taghizadeh SF (2021) Gynodioecy in two Iranian endemic thymes: the comparative study on their volatile compounds, cytological and morphological traits. Acta Physiol Plant 43:1–17

Mokhber-Dezfuli N, Saeidnia S, Gohari AR, Kurepaz-Mahmoodabadi M (2014) Phytochemistry and pharmacology of berberis species. Pharmacogn Rev 8:8

Nakamura Y, Watanabe S, Miyake N, Kohno H, Osawa T (2003) Dihydrochalcones: evaluation as novel radical scavenging antioxidants. J Agric Food Chem 51:3309–3312

Natto ZS, Siapco G, Jaceldo-Siegl K, Haddad EH, Sabaté J (2022) Food and nutrient displacement by walnut supplementation in a randomized crossover study. Nutrients 14:1017–1029. https://doi.org/10.3390/nu14051017
Özgen M, Saraçoğlu O, Gecer EN (2012) Antioxidant capacity and chemical properties of selected barberry (Berberis vulgaris L.) fruits. Hortic Environ Biotechnol 53:447–451
Pastell H, Putkonen T, Rita H (2019) Dietary fibre in legumes, seeds, vegetables, fruits and mushrooms: Comparing traditional and semi-automated filtration techniques. J Food Compos Anal 75:1–7
Penniston KL, Steele TH, Nakada SY (2007) Lemonade therapy increases urinary citrate and urine volumes in patients with recurrent calcium oxalate stone formation. Urology 70:856–860
Pinar H, Yahya HN, Erċışlı S, Coskun OF, Yaman M, Turgunbaev K, Uzun A (2021) Molecular characterization of barberry genotypes from Turkey and Kyrgyzstan. Erwerbs-Obstbau 63:403–407
Ponder A, Hallmann E (2020) The nutritional value and vitamin C content of different raspberry cultivars from organic and conventional production. J Food Compos Anal 87:103429
Pruthi N, Chawla GD, Yadav S (2008) Dyeing of silk with barberry bark dye using mordant combination. Indian J Nat Prod Resour 7(1):40–44
Rahimi-Madiseh M, Lorigoini Z, Zamani-Gharaghoshi H, Rafieian-Kopaei M (2017) Berberis vulgaris: specifications and traditional uses. Iran J Basic Med Sci 20:569
Rahmati M, Vercambre G, Davarynejad G, Bannayan M, Azizi M, Génard M (2015) Water scarcity conditions affect peach fruit size and polyphenol contents more severely than other fruit quality traits. J Sci Food Agric 95:1055–1065
Rahmati M et al (2018) Disentangling the effects of water stress on carbon acquisition, vegetative growth, and fruit quality of peach trees by means of the QualiTree model. Front Plant Sci 9:3
Ruiz A et al (2010) Polyphenols and antioxidant activity of calafate (Berberis microphylla) fruits and other native berries from Southern Chile. J Agric Food Chem 58:6081–6089
Saftner R, Polashock J, Ehlenfeldt M, Vinyard B (2008) Instrumental and sensory quality characteristics of blueberry fruit from twelve cultivars. Postharvest Biol Technol 49:19–26
Samadi S, Saharkhiz MJ, Azizi M, Samiei L, Ghorbanpour M (2020) Multi-walled carbon nanotubes stimulate growth, redox reactions and biosynthesis of antioxidant metabolites in Thymus daenensis celak. In Vitro Chemosphere 249:126069
Sarraf M, Beig Babaei A, Naji-Tabasi S (2019) Investigating functional properties of barberry species: an overview. J Sci Food Agric 99:5255–5269
Sellappan S, Akoh CC, Krewer G (2002) Phenolic compounds and antioxidant capacity of Georgia-grown blueberries and blackberries. J Agric Food Chem 50:2432–2438
Taghizadeh SF, Rezaee R, Davarynejad G, Karimi G, Nemati SH, Asili J (2018) Phenolic profile and antioxidant activity of Pistacia vera var. Sarakhs hull and kernel extracts: the influence of different solvents. J Food Meas Charact 12:2138–2144
Taghizadeh SF, Rezaee R, Mehmandoust M, Madarshahi FS, Tsatsakis A, Karimi G (2019) Coronatine elicitation alters chemical composition and biological properties of cumin seed essential oil. Microb Pathog 130:253–258
Taghizadeh SF, Rezaee R, Badibostan H, Karimi G (2020a) Aflatoxin B1 in walnuts: A probabilistic cancer risk assessment for Iranians. Toxicol Environ Chem 102(9):506–519. https://doi.org/10.1080/02772248.2020.1791868
Taghizadeh SF, Rezaee R, Badibostan H, Karimi G (2020b) Probabilistic carcinogenic and non-carcinogenic risk assessment of heavy metal ingestion through consumption of different walnut cultivars: An Iranian study. Environ Monit Assess 192:599–614. https://doi.org/10.1007/s10661-020-08551-4
Taghizadeh SF, Rezaee R, Boskabady M, Mashayekhi Sardoo H, Karimi G (2020c) Exploring the carcinogenic and non-carcinogenic risk of chemicals present in vegetable oils. Int J Environ Anal Chem. https://doi.org/10.1080/03067319.2020.1803848
Taghizadeh SF, Rezaee R, Mehmandoust M, Badibostan H, Karimi G (2020d) Assessment of in vitro bioactivities of Pis v 1 (2S albumin) and Pis v 2.0101 (11S globulin) proteins derived from pistachio (Pistacia vera L.). J Food Meas Charact 14:1054–1063
Taghizadeh SF, Azizi M, Rezaee R, Giesy JP, Karimi G (2021a) Polycyclic aromatic hydrocarbons, pesticides, and metals in olive: analysis and probabilistic risk assessment. Environ Sci Pollut Res 28(29):39723–39741. https://doi.org/10.1007/s11356-021-13348-0
Taghizadeh SF, Azizi M, Rezaee R, Madarshahi FS, Mehmandoust M, Karimi G, Asili J (2021b) Cytotoxic activity of cis-(E)-and trans-(Z)-spiroethers isolated from various Arnebia species S. Afr J Bot 142:114–123
Taguchi Y-H, Oono Y (2005) Relational patterns of gene expression via non-metric multidimensional scaling analysis. Bioinform 21:730–740
Tehranifar A, Zarei M, Nemati Z, Esfandiyari B, Vazifeshenas MR (2010) Investigation of physico-chemical properties and antioxidant activity of twenty Iranian pomegranate (Punica granatum L.) cultivars. Sci Hortic 126:180–185
Tural S, Koca I (2008) Physico-chemical and antioxidant properties of cornelian cherry fruits (Cornus mas L.) grown in Turkey. Sci Horticult 116:362–366
Wallace TC, Giusti MM (2008) Determination of color, pigment, and phenolic stability in yogurt systems colored with nonacylated anthocyanins from Berberis boliviana L. as compared to other natural/synthetic colorants. J Food Sci 73:C241–C248
Wang H, Conchou L, Bessière J-M, Cazals G, Schatz B, Imbert E (2013) Flower color polymorphism in Iris lutescens (Iridaceae): biochemical analyses in light of plant-insect interactions. Phytochem 94:123–134
Yaman M (2021) Evaluation of genetic diversity by morphological, biochemical and molecular markers in sour cherry genotypes. Mol Biol Rep. https://doi.org/10.1007/s11033-021-06941-6
Yaman M (2022) Determination of genetic diversity in european cranberrybush (Viburnum opulus L.) genotypes based on morphological, phytochemical and ISSR markers. Genet Resour Crop Evol 69:1889–1899
Yang L, Gou Y, Zhao T, Zhao J, Li F, Zhang B, Wu X (2012) Antioxidant capacity of extracts from calyx fruits of roselle (Hibiscus sabdariffa L.). Afr J Biotechnol 11:4063–4068
Yildiz H et al (2014) Some physicochemical characteristics, bioactive content and antioxidant characteristics of non-sprayed Barberry (Berberis vulgaris L.) fruits from Turkey. Erwerbs-Obstbau 56:123–129
Zarei M, Azizi M, Bashiri-Sadr Z (2010) Studies on physico-chemical properties and bioactive compounds of six pomegranate cultivars grown in Iran. J Food Technol 8:112–117
Zarei M, Azizi M, Bashir-Sadr Z (2011) Evaluation of physicochemical characteristics of pomegranate (Punica granatum L.) fruit during ripening. Fruits 66:121–129